DTNMAX (general vector)

Mini TN

Host specific constitutive promoter

TNP catalyzes the transposition of the mini TN and its contents.

Mini-TN inverted repeats from a transposon active in the target host organism.

MCS multiple cloning site for the introduction of gene libraries into the mini ISS1 transposon.

ORI – origin of replication for the desired cloning host organism.

Ori(cond) conditionally replicative origin of replication for target host organism.

in Line pTNWGS

Sel1, 2 markers for selection in cloning and target host organism.

SISS/MO

Host specific constitutive promoter

TN5 Tnp catalyzes the transposition of the mini TN917 and its contents.

tnD

Ori-ts temperature sensetive origin of replication for plasmid mainainance in target bacteria (gram positive or negative bacteria).

Mini TN5

Mini TN5 TN5 inverted repeats flanking a multiple cloning site into which gene libraries can be cloned.

CoIE1 origin of replication for plasmid maintainance in *E. coli.*

COIE

DINWGS

81/9/O

Kanr confers resistance to kanamycin to *E. coli.*

o sitive

Erm' confers resistance to erythromycin in Gram positive bacteria.

<u> Mini TN917</u>

Host specific

promoter - nisA
promoter for lactic acid
bacteria.

1917 TspR TspA catalyzes etransposition of the mini 1917 and its contents ansposase/resolvase)

PG+ temperature sensetive origin of replication for plasmid mainainance in Gram positive bacteria.

DTNWGS (SING)

MCS multiple cloning site for the introduction of gene libraries into the mini TN917 transposon.

<u>ග</u>

MCS

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tspR tpsA

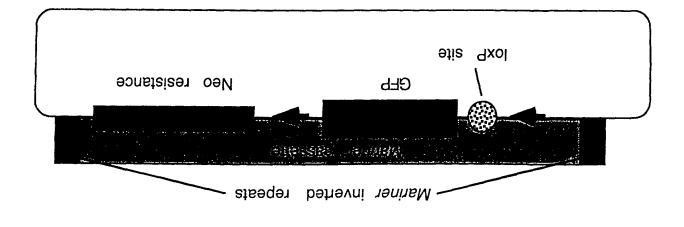
CoIE1 origin of replication for plasmid maintenance in *E. coli.*

Kanr confers resistance to kanamycin to *E. coli.*

erythromycin in Gram positive

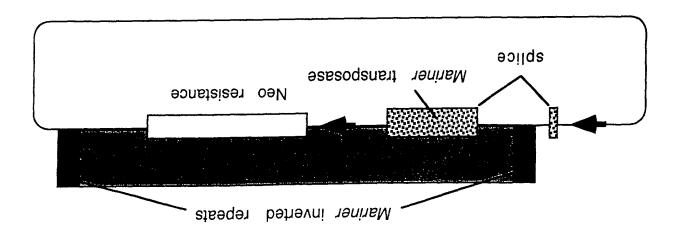
bacteria

Ermr confers resistance to



Mariner transposon for inserting loxP sites at loci with desirable expression properties

B



Efficient integration into mammalian cells using evolved Mariner transposons

Phenotypes by Whole Genome Shuffling (WGS) Methodology for Isolating Hosts with improved

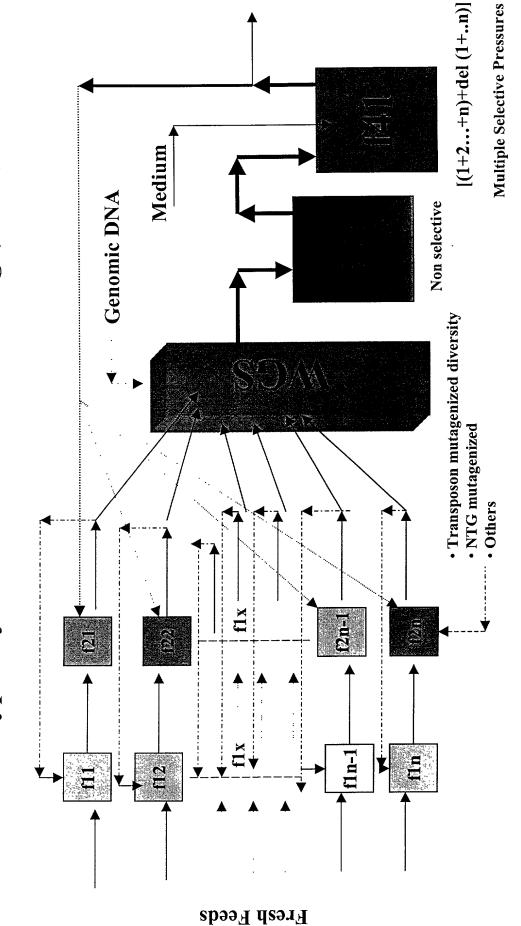
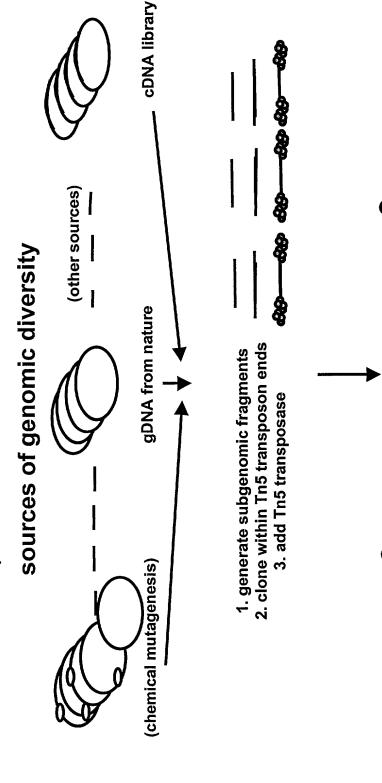


Figure 3

Shuffling of Genomes *In Vitro:* Formation of transposomes



* Complexes are actually circular as a result of transposase interaction

* "transposomes": complexes poised for

integration upon exposure to Mg++.

Figure 4A

Shuffling of Genomes In Vitro:

Breeding multiple donor genomes with a single acceptor genome

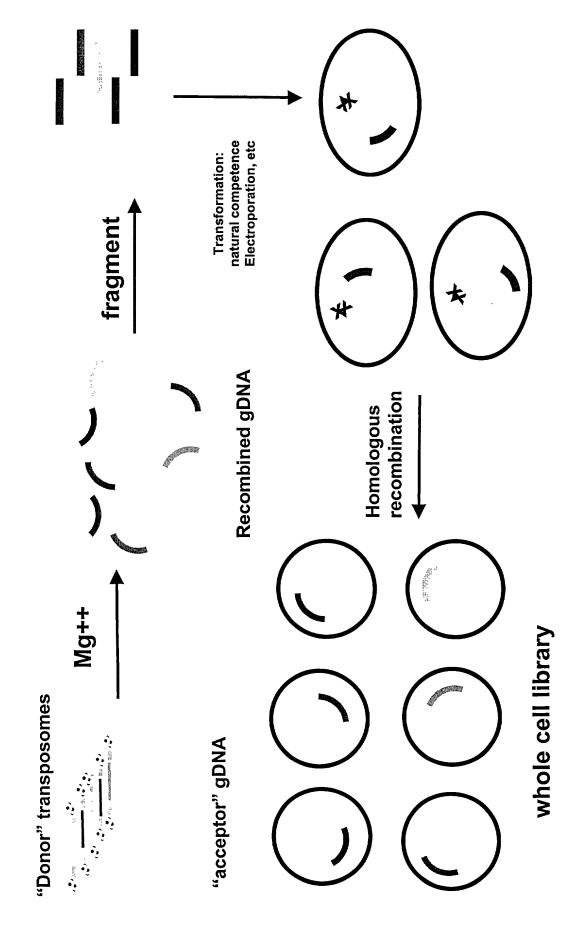


Figure 4B

Shuffling of Genomes In Vitro:

Breeding multiple donor genomes with multiple acceptor genomes

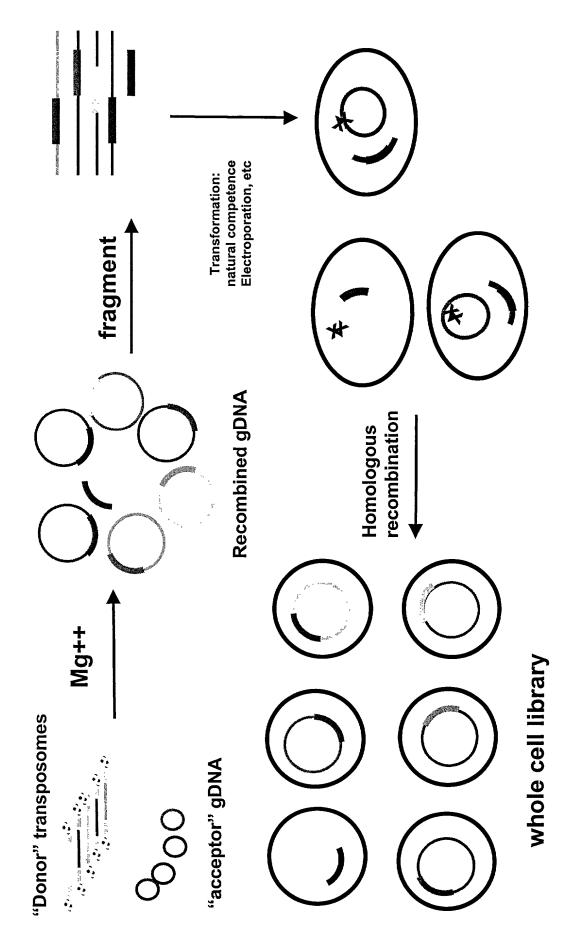


Figure 4C

Shuffling of Genomes In Vitro:

Split pool recursive in vitro recombination of multiple genomes natural competence Electroporation, etc Transformation: fragment Recombined gDNA Mg++ "Donor" transposomes split (A) is a measurement and the state of the st "acceptor" gDNA

Figure 4D

"acceptor" gDNA

"."Donor" transposomes

Deliver to cells